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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUL 25 1997

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Copper Triethanolamine Complex *(K-Tea)* and Copper Ethylenediamine Complex *(Komeen)*: Review of Toxicity Studies and Request for a waiver from Conducting Additional Testing

FROM: Sanjivani Diwan, Ph.D. Sanjivani Diwan 7/16/97
Review Section I, Toxicology Branch II
Health Effects Division (7509C)

TO: Kathryn Davis/Dean Monos/PM-52
Accelerated Reregistration Branch
Special Review and Reregistration Division
(7508W)

THROUGH: Jess Rowland, M.S., Acting Head 7/16/97
Review Section I, Toxicology Branch II
Health Effects Division (7509C)
and
Yiannakis M. Ioannou, Ph.D.
Chief, Toxicology Branch II
Health Effects Division (7509C) J.M. Ioannou 7/24/97

P. C. CODE: 024403; 024407
SUBMISSION NO.: S512801; S512802; S512828
DP BARCODE: D230571; D230574; D230596
MRID NUMBER: 44127506 & 44127507

Registrant: Griffin Corporation, Valdosta, GA.

Action Requested: Toxicology Branch II has been asked to review 12- Day Dermal Range-Finding and Developmental Toxicity Screening Studies of K-Tea and Komeen submitted by Griffin Corporation, GA. The Registrant is requesting a waiver from conducting full studies on these chemicals to support their reregistration.

Recommendation: Toxicology Branch II has determined that the dermal toxicity studies on K-Tea and Komeen are Acceptable and adequate for reregistration purposes since use of higher dose levels would compromise health of the animals. However, the Registrant should repeat the developmental toxicity studies with K-Tea and Komeen since only two dose levels were tested instead of three dose levels as recommended in the Subdivision F

Guidelines. In addition, full evaluation of developmental parameters should be made to meet reregistration requirements.

Background: In a letter dated July 3, 1996 (by S. Diwan to K. Davis and D. Monos, Special Review and Reregistration Division), the Agency requested the submission of dermal range-finding as well as developmental toxicity screening studies upon completion so that the Agency can review and if necessary recommend dose selection levels. Registrant has now submitted results of completed studies. Registrant contended that the results of 12 days dermal application studies are sufficient to assess the potential human risk from dermal exposure to K-Tea and Komeen. Also the highest doses of these compounds were adequate for evaluation of developmental effects as they produce maternal toxicity (i.e., decreased body weight gain and food consumption). The lowest doses used in these studies were nontoxic. Additionally, the use patterns for both compounds limits the potential human exposure especially women of childbearing age. Thus, the Registrant claims that the absence of developmental toxicity at maternally toxic doses coupled with lack of human exposure suggests that full developmental studies are not warranted.

Agency's Response: Toxicology Branch II has reviewed the dermal range-finding studies on K-Tea and Komeen and found them acceptable for the purposes they were conducted. The available data confirm the findings that both Komeen and K-Tea cause skin irritation without appreciable systemic toxicity. The developmental toxicity studies on K-Tea and Komeen are inadequate for reregistration purposes for the reasons stated below; the summaries of these studies follow:

Review of Toxicology Data

Dermal Range-Finding Studies (Guideline §82-2; MRID # 44127507):

- 12-Day dermal toxicity study on K-Tea (MRID # 44127507)

  Three groups of Sprague-Dawley Crl:CD BR rats (5 sex/group) received topical application of undiluted test article at dosages of either 0, 100 or 1000 mg/kg/day for six hours per day for 12 days.

  Treatment-related dermal irritation was noted in animals at ≥100 mg/kg/day. Erythema, edema, fissuring, desquamation and eschar were noted in these dose groups, while exfoliation and/or clear exudate were limited to 1,000 mg/kg/day group females. No evidence of systemic toxicity was observed at either dose levels.

The systemic toxicity LOEL is >1000 mg/kg/day for males and females; the systemic toxicity NOEL is ≥1000 mg/kg for males and females.

The dermal toxicity LOEL is 100 mg/kg/day for males and females; the dermal toxicity NOEL is <100 mg/kg/day for males and females.

The study is classified as Acceptable (Nonguideline) for the purposes for which it was intended as a range-finding study. However, it is not a required guideline study.

- 12-Day dermal toxicity study on Komeen (MRID # 44127507)

Three groups of Sprague-Dawley Crl:CD BR rats (5 sex/group) were treated topically with undiluted test article at dosages of either 0, 100 or 1000 mg/kg/day for six hours per day for 12 days.

Treatment-related dermal irritation was noted in animals at ≥100 mg/kg/day. Erythema, edema, fissuring, desquamation and eschar were noted in these dose groups, while exfoliation, corrosion and/or clear exudate were limited to the 1,000 mg/kg/day group. No evidence of systemic toxicity was observed at either dose levels.

The systemic toxicity LOEL is >1000 mg/kg/day for males and females; the systemic toxicity NOEL is ≥1000 mg/kg for males and females.

The dermal toxicity LOEL is 100 mg/kg/day for males and females; the dermal toxicity NOEL is <100 mg/kg/day for males and females.

The study is classified as Acceptable (Nonguideline) for the purposes for which it was intended as a range-finding study. However, it is not a required guideline study.

- Developmental Toxicity of K-Tea in Rat (MRID No: 44127506)

K-Tea (8.2% Copper as a.i.) was administered in water (5 mL/kg) to three groups of twelve female Sprague-Dawley rats by gavage at dose levels of 0, 100, or 700 mg/kg/day from gestational days 6-15, inclusive.

One female from 700 mg/kg/day dose group died on GD 10. Transient reduction in body weight gain accompanied by decrease in food consumption was noted during first two days of dosing in the 700 mg/kg/day dose group. **The maternal LOEL is 700 mg/kg/day based on transient decrease in body weight gains and food consumption. The maternal NOEL is 100 mg/kg/day.**

Developmental toxicity, observed at 700 mg/kg/day, was manifested as increased incidence of postimplantation loss. **The LOEL for developmental toxicity is 700 mg/kg/day, based on increased postimplantation loss. The developmental NOEL is 100 mg/kg/day.**

This study is classified as **Acceptable** (nonguideline) for the purposes for which it was intended as a screening study. However, the study should be repeated using an intermediate dose level. Since only two dose levels were used, additional testing at an intermediate dose level would be helpful in assessing the dose-response. Also a detailed examination of fetuses should be conducted to fully investigate the developmental potential of the test compound.

- Developmental Toxicity of K-Tea in Rat (MRID No: 44127506)

Komeen (8.0% Copper as a.i.) was administered in water (5 mL/kg) to three groups of twelve female Sprague-Dawley rats by gavage at dose levels of 0, 100, or 500 mg/kg/day from gestational days 6-15, inclusive.

Maternal toxicity observed at 500 mg/kg/day, was manifested as transient reduction in body weight gain accompanied by decrease in food consumption during first day of dosing. However, no frank toxicity was observed. **The maternal NOEL/LOEL is 500 mg/kg/day, based on transient decrease in body weight gain and food consumption. The maternal NOEL is 100 mg/kg/day.**

No developmental toxicity was observed. **The NOEL for developmental toxicity is 3500 mg/kg/day. The developmental LOEL was not established.**

This study is classified as **Unacceptable** (nonguideline) because the highest dose failed to produce frank maternal toxicity. The lack of developmental toxicity at 500 mg/kg/day, based on limited observations, does not guarantee safe exposure at >500 mg/kg/day. The study should be repeated at two higher dose levels including the limit dose (1,000 mg/kg/day) to determine

the potential developmental toxicity and to allow assessment of dose-response. A detailed fetal examination should be conducted to fully investigate the developmental effects of the test compound.

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KOMEEN Range-Finding Dermal Study

Reviewed by: Sanjivani Diwan, Ph.D. Sanjivani S. Diwan Date: 6-18-97
Review Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Alan C. Levy, Ph.D. Alan C. Levy Date: 6-18-97
Review Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE:	12-Day Dermal Toxicity/Rats
EPA I.D. NUMBERS:	DP BARCODE: D230596 P.C. CODE: 024407 MRID NUMBER: 44127507 SUBMISSION No.: S512828
TEST MATERIAL:	Komeen CAS No.: 13426-91-0 Synonym: Bisethylendiamine copper chelate; Copper ethylenediamine complex
STUDY NUMBER:	WIL-157013
TESTING FACILITY:	WIL Research Laboratories, Inc., Ashland, OH
SPONSOR:	Griffin Corporation, Valdosta, GA
TITLE OF REPORT:	A 12-Day Dermal Range-Finding Study of K-Tea and Komeen in Rats
AUTHOR:	G.R. Kiplinger
REPORT ISSUED:	September 23, 1996

EXECUTIVE SUMMARY: In this 12-day dermal toxicity study (MRID # 44127507), 3 groups of Sprague-Dawley Crl:CD BR rats (5 sex/group) were treated topically with undiluted test article at dosages of either 100 or 1000 mg/kg/day for six hours per day for 12 days. The test material was applied undiluted daily at a volume-dosage of 0.08 and 0.82 mL/kg body weight. A concurrent control group received 0.9% saline (0.86 mL/kg/day) in a similar manner.

A dose-related increase in dermal irritation was noted in animals at ≥100 mg/kg/day. Erythema, edema, fissuring, desquamation and eschar were noted in these dose groups, while exfoliation,, corrosion and clear exudate were limited to the 1,000 mg/kg/day group. No evidence of systemic toxicity was observed at either dose levels.

The systemic toxicity LOEL is >1,000 mg/kg/day for males and females based on lack of toxicity; the NOEL is ≥1000 mg/kg.

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The dermal toxicity LOEL is 100 mg/kg/day for males and females; the dermal toxicity NOEL is <100 mg/kg/day for males and females.

The study is classified as Acceptable for the purposes for which it was intended as a range-finding study. However, it is not a required guideline study.

I. MATERIALS

A. Test Material

Name: Komeen
Synonym:Copper ethylenediamine complex
Purity: 8% copper (doses not adjusted for purity)
Lot Number: 1150007149
Description: Dark blue liquid
Storage Conditions: Under ambient conditions

B. Vehicle: 0.9% sodium chloride

C. Administration: dermal application

D. Test Animals:

Species: Rat
Species: Sprague-Dawley Crl:CD BR
Source: Charles River Breeding Laboratories, Inc., Portage, MI.
Age: Males - approximately 8 weeks; Females - approximately 11 weeks at initiation of dosing
Weight: Males - 244-295 g; Females - 212–249 g at initiation of dosing
Housing: Individually in wire-mesh cages
Environmental Conditions:
Temperature: 71.5-72.2°F
Relative humidity: 22.6–45.3%
Photoperiod: 12 hours light/dark
Air changes: Not reported
Food and Water: Purina Certified Rodent Chow #5002 and water *ad libitum*
Acclimation Period: 13 days

II. METHODS

A. Preparation of Dosing Substance

An adequate amount of the test article was dispensed into labelled containers for each treated group. Doses were based on the density of the test article. Preparations were stirred throughout the dose administration.

B. Dosage and Administration

Dosage Groups

The animals were assigned by computer randomization procedure to the following treatment groups:

Table 1. Animal Assignment

Test Group	Dose (mg/kg/day)	# Males	# Females
1 (Control)	0	5	5
2	100	5	5
3	1,000	5	5

Dosages were selected by the sponsor; no rationale was provided. The study was extended from 5 days of dosing to 12 days of dosing because very little evidence of skin irritation was present after 5 days of dosing.

Administration

One day prior to the initiation of dosing, the hair of each rat was clipped from the dorsal area extending from shoulder to rump. The test area constituted approximately 20-25% of the total body surface. The procedure was repeated weekly thereafter. The test material or vehicle was applied evenly over the clipped area of each animal once daily, six hours per day for twelve days at dose volumes of 0.86, 0.08 and 0.82 mL/kg for control, 100 and 1,000 mg/kg/day groups, respectively; the test material was applied undiluted using a glass rod. The individual doses were calculated based on the most current individual body weight recorded prior to test article application. A gauze patch was held in place by a semi-occlusive wrap and secured in place by tape. The animals were fitted with a plastic Elizabethan restraint collar to prevent ingestion of the test substance. Approximately 6 hours after the application, the site was washed with wet disposable towels to remove residual test article. Clinical findings including changes in the skin, fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous system function, somatomotor activity and behavior patterns were recorded daily. Skin reactions were scored using Draize's method. All animals were sacrificed after 12 days of treatment.

C. Experimental Design

The study protocol required the following observations and examinations at the indicated times or frequencies:

Signs of dermal irritation -once daily
Signs of mortality and morbidity - twice daily
Systemic toxicity - at least twice daily (before dosing and after dosing); and a detailed examination weekly
Body weights - prior to treatment, and twice thereafter (on days 5 and 12); body weights were calculated for each interval
Food consumption - prior to treatment, and twice thereafter (on days 5 and 12)
Gross necropsy - all animals

D. Pathological Examination

At necropsy skin and all gross lesions were preserved in 10% neutral buffered formalin for possible microscopic examination.

E. Statistical Analyses

The following procedures were utilized in analyzing the numerical data:

- All analyses involving comparison of the treatment groups with the control group by sex - two-tailed tests

- mean body weight, body weight change, and food consumption - one-way analysis of variance followed by Dunnett's test

F. Compliance

Signed statements of Quality Assurance and compliance with Good Laboratory Practice regulations were submitted by the testing facility. The sponsor submitted a statement claiming no data confidentiality.

III. RESULTS

A. Administered Dosage

The undiluted test article was applied to each animal at a dose volume of 0.86, 0.08 and 0.82 mL/kg for control, 100 and 1,000 mg/kg/day groups, respectively.

B. Mortality/Clinical Observations and Systemic Toxicity

There were no deaths nor signs of systemic toxicity observed during the study. Treatment-

related increase in dermal irritation was noted at the test site. At 100 mg/kg/day, desquamation and focal eschar were noted early in the study (study days 3 or 4) while nonfocal eschar and fissuring were observed later in the study (study day 6 and later). At 1,000 mg/kg/day, dermal findings noted prior to study day 6 included desquamation, fissuring, eschar (focal and nonfocal) and clear exudate; exfoliation and corrosion were observed after study day 7. Erythema, edema, fissuring, desquamation and eschar (both focal and nonfocal) were noted in both treatment groups while more severe effects such as exfoliation and clear exudate were seen in the 1,000 mg/kg/day group males and females with corrosion noted in only two females. These findings are summarized in Table 2.

C. Body Weight and Body Weight Gain

Mean body weights and body weight gains of the treated animals did not differ significantly differ from those of the controls.

D. Food Consumption and Food Conversion Ratio

There were no significant differences between the treated and control groups in mean daily food consumption (g/animal/day).

E. Gross Pathology

Test article-related gross skin lesions noted at the application site were limited to scabbing (males: 1/5, 2/5; females: 1/5, 2/5 at 100 and 1,000 mg/kg/day, respectively), thickened skin (3/5 males, 1/5 females at 1,000 mg/kg/day) and abscess (1/5 females at 1,000 mg/kg/day).

F. Necropsy Findings

There were no test article-related lesions seen at the scheduled necropsy.

G. Conclusion from Study Report

The study report concludes that the topical application of undiluted Komeen caused localized irritation at all dose levels. Although erythema, edema, fissuring, desquamation and eschar (both focal and nonfocal) were observed at the application site in all treated groups; exfoliation, corrosion and/or clear exudate were limited to the 1,000 mg/kg/day group females. Gross tissue changes were noted at the application site in both the treated groups. No evidence of systemic toxicity was observed at either dose levels. The LOEL for systemic toxicity is $>1,000$ mg/kg/day (Limit dose) for male and female rats based on the lack of evidence of systemic toxicity; the No Observed Effect Level (NOEL) is $\geq 1,000$ mg/kg/day. The LOEL for dermal toxicity is 100 mg/kg/day for male and female rats based on clinical signs of dermal toxicity; the NOEL is <100 mg/kg/day.

H. STUDY DEFICIENCY

No deficiencies were noted.

IV. DISCUSSION/CONCLUSIONS

In this 12-day dermal toxicity range-finding study, five Sprague-Dawley CD rats/sex/group were treated topically with dosages of either 0, 100 or 1,000 mg/kg of Komeen six hours per day for 12 days. Dose-related increases in erythema, edema, fissuring, desquamation and eschar (both focal and nonfocal) were observed at the application sites in both treated groups. Exfoliation and clear exudate were observed in both sexes while corrosion was noted only in two females at 1,000 mg/kg/day. Gross lesions observed at the test sites in both the treatment groups included scabbing while thickened skin and abscess were seen only at 1,000 mg/kg/day. There were no differences between the control and treated groups in any of the other parameters measured. No signs of systemic toxicity were noted in the treated animals.

The systemic toxicity LOEL is >1000 mg/kg/day for males and females; the systemic toxicity NOEL is ≥1000 mg/kg/day for males and females.

The dermal toxicity LOEL is 100 mg/kg/day for males and females; the dermal toxicity NOEL is <100 mg/kg/day for males and females.

Table 2. Dermal findings

Sex	Male (5/group)			Females (5/group)		
Dose (mg/kg/day)	0	100	1,000	0	100	1,000
Erythema						
-Very slight	0	5	5	0	5	5
-Slight	0	0	3	0	1	4
-Severe	0	1	4	0	2	2
Edema						
-Very slight	0	1	4	0	3	3
-Slight	0	1	3	0	0	2
-Moderate	0	0	1	0	0	2
Other						
-Fissuring	0	1	4	0	1	2
-Desquamation	3	5	5	3	5	5
-Eschar	0	1	4	0	2	2
-Focal Eschar	0	4	5	0	4	3
-Exfoliation	0	0	3	0	0	2
-Corrosion	0	0	0	0	0	2
-Clear exudate	0	0	2	0	0	2

a Data extracted from Table 2, pages 36-38 of the study report

K-TEA Range-Finding Dermal Study

Reviewed by: Sanjivani Diwan, Ph.D. Sanjivani B. Diwan Date: 6-18-97
Review Section I, Toxicology Branch II (7509C)
Secondary Reviewer: Alan C. Levy, Ph.D. Alan C. Levy ,Date: 6-18-97
Review Section I, Toxicology Branch II (7509C)

DATA EVALUATION REPORT

STUDY TYPE:	12-Day Dermal Toxicity/Rats
EPA I.D. NUMBERS:	DP BARCODE: D230596 P.C. CODE: 024403 MRID NUMBER: 44127507 SUBMISSION No.: S512828
TEST MATERIAL:	K-Tea CAS No.: 31089-39-1 Synonym: Copper triethanolamine complex
STUDY NUMBER:	WIL-157013
TESTING FACILITY:	WIL Research Laboratories, Inc., Ashland, OH
SPONSOR:	Griffin Corporation, Valdosta, GA
TITLE OF REPORT:	A 12-Day Dermal Range-Finding Study of K-Tea and Komeen in Rats
AUTHOR:	G.R. Kiplinger
REPORT ISSUED:	September 23, 1996

EXECUTIVE SUMMARY: In this 12-day dermal toxicity study (MRID # 44127507), 3 groups of Sprague-Dawley Crl:CD BR rats (5 sex/group) were treated topically with undiluted test article at dosages of either 100 or 1000 mg/kg/day for six hours per day for 12 days. The test material was applied undiluted daily at a volume-dosage of 0.09 and 0.86 mL/kg body weight. A concurrent control group received 0.9% saline (0.86 mL/kg/day) in a similar manner.

Dose-related increase in dermal irritation was noted in animals at ≥ 100 mg/kg/day. Erythema, edema, fissuring, desquamation and eschar were noted in these dose groups, while exfoliation, and clear exudate were limited to the 1,000 mg/kg/day group. No evidence of systemic toxicity was observed at either dose levels.

The LOEL for systemic toxicity is >1,000 mg/kg/day for males and females based on lack of systemic toxicity; the NOEL is ≥1000 mg/kg.

The dermal toxicity LOEL is 100 mg/kg/day for males and females; the NOEL is <100 mg/kg/day.

The study is classified as Acceptable for the purposes for which it was intended as a range-finding study. However, it is not a required guideline study.

I. MATERIALS

A. Test Material

Name: K-Tea
Synonym:Copper triethanolamine complex
Purity: 8.2% copper (doses not adjusted for purity)
Lot Number: 0950019159
Description: Dark blue liquid
Storage Conditions: Under ambient conditions

B. Vehicle: 0.9% sodium chloride

C. Administration: Dermal application

D. Test Animals:

Species: Rat
Species: Sprague-Dawley Crl:CD BR
Source: Charles River Breeding Laboratories, Inc., Portage, MI.
Age: Males - approximately 8 weeks; Females - approximately 11 weeks at initiation of dosing
Weight: Males - 244-295 g; Females - 212–249 g at initiation of dosing
Housing: Individually in wire-mesh cages
Environmental Conditions:
Temperature: 71.5-72.2°F
Relative humidity: 22.6–45.3%
Photoperiod: 12 hours light/dark
Air changes: Not reported
Food and Water: Purina Certified Rodent Chow #5002 and water *ad libitum*
Acclimation Period: 13 days

II. METHODS

A. Preparation of Dosing Substance

An adequate amount of the test article was dispensed into labelled containers for each treated group. Doses were based on the density of the test article. Preparations were stirred throughout the dose administration.

B. Dosage and Administration

Dosage Groups

The animals were assigned by computer randomization procedure to the following treatment groups:

Table 1. Animal Assignment

Test Group	Dose (mg/kg/day)	# Males	# Females
1 (Control)	0	5	5
2	100	5	5
3	1,000	5	5

Dosages were selected by the sponsor; no rationale was provided. The study was extended from 5 days of dosing to 12 days of dosing because very little evidence of skin irritation was present after 5 days of dosing.

Administration

One day prior to the initiation of dosing, the hair of each rat was clipped from the dorsal area extending from shoulder to rump. The test area constituted approximately 20-25% of the total body surface. The procedure was repeated weekly thereafter. The test material or vehicle were applied evenly over shaved area of each animal once daily, six hours per day for twelve days at dose volume of 0.86 and 0.09 mL/kg (for control and 100 mg/kg/day groups, respectively) or 0.86 mL/kg for the 1,000 mg/kg/day group; the test material was applied undiluted using a glass rod. The individual doses were calculated based on the most current individual body weight recorded prior to test article application. A gauze patch was held in place by semi-occlusive wrap and secured by tape. The animals were fitted with a plastic Elizabethan restraint collar to prevent ingestion of the test substance and/or wrappings. Approximately 6 hours after the application, the application site was washed with wet disposable towels to remove residual test article. Clinical findings including changes in the skin, fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous system function, somatomotor activity and behavior patterns were recorded daily. Skin reactions were scored using Draize's method. All animals were sacrificed after 12 days of treatment.

C. Experimental Design

The study protocol required the following observations and examinations at the indicated times or frequencies:

Signs of dermal irritation -once daily
Signs of mortality and morbidity - twice daily
Systemic toxicity - at least twice daily (before dosing and after dosing); and a detailed examination weekly
Body weights - prior to treatment, and twice thereafter (on days 5 and 12); body weights were calculated for each interval
Food consumption - prior to treatment, and twice thereafter (on days 5 and 12)
Gross necropsy - all animals

D. Pathological Examination

At necropsy skin and all gross lesions were preserved in 10% neutral buffered formalin for possible microscopic examination.

E. Statistical Analyses

The following procedures were utilized in analyzing the numerical data:

- All analyses involving comparison of the treatment groups with the control group by sex - two-tailed tests

- mean body weight, body weight change, and food consumption - one-way analysis of variance followed by Dunnett's test

F. Compliance

Signed statements of Quality Assurance and compliance with Good Laboratory Practice regulations were submitted by the testing facility. The sponsor submitted a statement claiming no data confidentiality.

III. RESULTS

A. Administered Dosage

The undiluted test article was applied to each animal at a dose volume of 0.09 mL/kg for 100 mg/kg/day groups or 0.86 mL/kg for the 1,000 mg/kg/day group.

B. Mortality/Clinical Observations and Systemic Toxicity

There were no deaths nor signs of systemic toxicity observed during the study. Dose-related increase in dermal irritation was noted at the test site. At 100 mg/kg/day, desquamation was noted early in the study (study day 3 or 4), while focal or nonfocal eschar and fissuring (females) were observed later in the study (study day 5 or later). At 1,000 mg/kg/day, dermal findings noted included desquamation, fissuring, eschar (focal and nonfocal) from study day 3. Exfoliation and clear exudate were observed in females on study day 7 and 9, respectively. Desquamation was noted in controls while erythema, edema, fissuring, desquamation and eschar (both focal and nonfocal) were noted in both treatment groups. More severe effects such as exfoliation and clear exudate were seen only in 1,000 mg/kg/day group females. These findings are summarized in Table 2.

C. Body Weight and Body Weight Gain

Mean body weights and body weight gains of the treated animals did not significantly differ from those of the controls.

D. Food Consumption and Food Conversion Ratio

There were no significant differences between the treated and control groups in mean daily food consumption (g/animal/day).

E. Gross Pathology

Test article-related gross skin lesions noted at the application site or in the neck region were limited to scabbing (males: 1/5, 3/5; females: 3/5, 3/5 at 100 and 1,000 mg/kg/day, respectively).

F. Necropsy Findings

There were no test article-related lesions seen at the scheduled necropsy.

G. Conclusion from Study Report

The study report concludes that the topical application of undiluted K-Tea caused localized irritation at both dose levels. Although erythema, edema, fissuring, desquamation and eschar (both focal and nonfocal) were observed at the application site in both treated groups. Exfoliation and/or clear exudate were limited to 1,000 mg/kg/day group females. No evidence of systemic toxicity was observed at either dose levels. The LOEL for systemic toxicity is >1,000 mg/kg/day (Limit dose) for male and female rats based on the lack of evidence of systemic toxicity; the No Observed Effect Level (NOEL) is ≥1,000 mg/kg/day. The LOEL for dermal toxicity is 100 mg/kg/day for male and female rats based on clinical signs of dermal toxicity; the NOEL is <100 mg/kg/day.

H. STUDY DEFICIENCY

No deficiencies were noted.

IV. DISCUSSION/CONCLUSIONS

In this 12-day dermal toxicity range-finding study, five Sprague-Dawley CD rats/sex/group were treated topically with dosages of either 0, 100 or 1,000 mg/kg of K-Tea six hours per day for 12 days. Dose-related increases in erythema, edema, fissuring, and focal or nonfocal eschar were observed at the application sites in one or both sexes. Exfoliation and clear exudate were observed only in females at 1,000 mg/kg/day. There were no differences between the control and treated groups in any of the other parameters measured. No signs of systemic toxicity were noted in the treated animals.

The systemic toxicity LOEL is >1000 mg/kg/day for males and females; the systemic toxicity NOEL is ≥1000 mg/kg/day for males and females.

The dermal toxicity LOEL is 100 mg/kg/day for males and females; the dermal toxicity NOEL is <100 mg/kg/day for males and females.

Table 2. Dermal findings

Sex	Male (5/group)			Females (5/group)		
Dose (mg/kg/day)	0	100	1,000	0	100	1,000
Erythema						
-Very slight	0	4	5	0	5	5
-Slight	0	1	0	0	1	1
-Severe	0	0	1	0	4	4
Edema						
-Very slight	0	1	1	0	3	4
-Slight	0	0	0	0	3	3
-Moderate	0	0	0	0	0	0
Other						
-Fissuring	0	0	1	0	3	3
-Desquamation	3	5	5	3	5	5
-Eschar	0	0	1	0	4	4
-Focal Eschar	0	4	5	0	3	2
-Exfoliation	0	0	0	0	0	2
-Clear exudate	0	0	0	0	0	3

a Data extracted from Table 2, pages 36-38 of the study report WIL-157013

012283

Primary Review by: Sanjivani B. Diwan, Ph.D. Date 7/16/97
Review Section I, Toxicology Branch II/HED (7509C)
Secondary Review by: Stephen C. Dapson, Ph.D. Date 7/16/97
Review Section I, Toxicology Branch II/HED (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Teratology - Developmental Toxicity/Rat

DP BARCODE: D230571; D230574

P. C. CODE: 024403

MRID No: 44127506

SUBMISSION No.: S512801; S512802

TEST MATERIAL: K-TEA

CAS No.: 31089-39-1

SYNONYMS: Copper triethanolamine complex

STUDY REPORT NUMBER: WIL-157014

SPONSOR: Griffin Corporation, Valdosta, GA

TESTING FACILITY: WIL Research Laboratories, Inc., Ashland, OH

TITLE OF REPORT: A Developmental Toxicity Screening Study of K-Tea and Komeen in Rats

AUTHOR: D. G. Stump

REPORT ISSUED: August 9, 1996

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID# 44127506) K-Tea (8.2% Copper as a.i.) was administered in water (5 mL/kg) to two groups of twelve female Sprague-Dawley rats by gavage at dose levels of 0, 100, or 700 mg/kg/day from gestational days 6-15, inclusive.

One female from 700 mg/kg/day dose group died on GD 10. Transient reduction in body weight gain accompanied by decrease in food consumption was noted during first two days of dosing in the 700 mg/kg/day dose group. **The maternal LOEL is 700 mg/kg/day based on clinical signs, decrease in body weight gains and food consumption. The maternal NOEL is 100 mg/kg/day.**

Developmental toxicity, observed at 700 mg/kg/day, was manifested as significantly increased incidence of postimplantation loss. **The LOEL for developmental toxicity is 700 mg/kg/day, based on increased postimplantation loss. The developmental NOEL is 100 mg/kg/day.**

This study is classified as **Acceptable** (nonguideline) for the purposes which it was intended as a range-finding study. However, the study should be repeated using an intermediate dose level to asses the dose-response. A detailed fetal examination should be conducted to fully investigate the developmental potential of the test compound.

COMPLIANCE: The signed statements of No Data Confidentiality Claim, Compliance with EPA, OECD and Japanese MAFF GLPs as well as Quality Assurance statements were provided. No Flagging Criteria statement was provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: K-Tea
   Synonym: Copper triethanolamine complex
   Purity: 8.2% copper
   Lot/Batch Number: 0950019159
   Description: Dark blue liquid
   Storage Conditions: Under ambient conditions

2. Vehicle: Deionized water

3. Test Animals: Rat
   Species: Sprague-Dawley Crl:CD®BR
   Source: Charles River Breeding Laboratories, Inc., Portage, MI.
   Age: 12.5 weeks at initiation of dosing
   Weight: Females - 226-276 g at initiation of dosing
   Housing: Individually in wire-mesh cages
   Environmental Conditions:
   Temperature: target of 72±4°F
   Relative humidity: target of 30-70%
   Photoperiod: 12 hours light/dark
   Air changes: Not reported
   Food and Water: Purina Certified Rodent Chow #5002 and water *ad libitum*
   Acclimation Period: 13 days
   Males used: Untreated, sexually mature males from the same source and strain

B. PROCEDURES AND STUDY DESIGN:

This study was designed to assess developmental toxicity of K-TEA when administered daily by gavage to rats on gestation days 6 through 15, inclusive.

1. Mating: Following acclimation, females were mated with males of the same strain and source. Females were checked daily for the presence of vaginal sperm or a copulatory plug. The day on which mating was confirmed was designated day 0 of gestation.

2. Animal Assignment and dose selection is presented in Table 1. Animals were consecutively assigned to three

groups containing twelve rats each by the randomization procedure as shown below.

TABLE 1. Animal Assignment

Test Group	Dose Level (mg/kg/day)	Number Assigned per Group
Control[a]	0	12
Mid-dose	100	12
High-dose	700	12

a One control female was inadvertently dosed on GD 16.

3. Dose Selection Rationale: The dose levels were selected based upon the results of preliminary five-day toxicity study by the sponsor. In this study K-Tea was administered to groups of two nonpregnant female rats at oral doses of 50, 100, 200, 400, 600 and 800 mg/kg/day K-Tea for five consecutive days; one female at 800 mg/kg/day died on day 3. This female exhibited lethargy, pale eyes, lacrimation, ptosis, brown, green, red staining/mating on various parts of body and decreased defecation, soft stool and green mucoid feces. Mean body weights and body weight gains in the treated groups were comparable to control group values.

4. Dosing: All doses were administered by gavage on GD 6-15 in a volume of 5 ml/kg of body weight/day prepared twice during the study. The appropriate amounts of the test material was weighed into precalibrated container to the calibrated mark. Sufficient amount of deionized water, was added to the container. The preparations were stirred on a magnetic stir plate throughout the sampling and dosing procedures. The formulations were divided into aliquots for daily dose administration and stored at room temperature. The doses were not adjusted for purity of the test compound. The individual dosages were based on the most recently recorded body weights to provide the correct mg/kg dose. Prior to dosing, one set of each sample,

collected from the top, middle and bottom portions of the formulations, was analyzed to determine the homogeneity. The stability of the second set of samples was determined over 8 day period.

C. OBSERVATIONS:

1. Maternal Observation and Evaluations - Rats were observed for moribundity and mortality twice daily. They were checked for signs of toxicity approximately one hour following dosing. Individual clinical observations were recorded daily prior to dosing and during the dosing period (GD 0-20). Body weights were recorded on gestation day (GD) 0, 6 to 16 (daily) and 20; group mean body weight changes were calculated for each corresponding intervals and also for intervals 6-9, 9-12, 12-16, 6-16 and 0-20. Food consumption was recorded for the following periods: GD 0, 6-16 (daily) and 20. Examinations at sacrifice consisted of:

- Gross pathology observations for evidence abnormalities
- Individual uterine weights
- Number of corpora lutea
- Number of implantation sites; the uteri from apparently nonpregnant animals were placed in 10% ammonium sulfide solution using the Salewski (1964) technique to detect early implantation loss
- Numbers of resorptions (early and late) and live and dead fetuses
- Number and distribution of fetuses in each uterine horn

2. Fetal Evaluations - The fetuses were examined in the following manner:

- Individual fetal weight and sex
- External anomalies
- Crown-rump measurements for late resorptions

3. Historical control: These were provided to allow comparison with concurrent controls.

D. STATISTICAL ANALYSIS: The following methods were used.

- Maternal and fetal body weight and body weight change, uterine weights and net body weight changes, food consumption, number of corpora lutea, total number of implantations and viable fetuses--one-way ANOVA with Dunnett's test

- Early and late resorptions, dead fetuses, post-implantation losses--Mann-Whitney U-test

- Fetal sex ratios--Chi-square test with Yates' correction factor

- Malformations and variations-- Fischer's Exact test

II. RESULTS

A. TEST MATERIAL ANALYSES

The concentration analysis for the first set of samples for the two dose groups indicated mean values within ±3% of the nominal concentration (99% and 103% at 100 and 700 mg/kg/day, respectively). Stability analyses conducted on two sets of test samples revealed values within ±7% of target (92.8%-97%), thus showing no degradation. The test compound was homogeneously distributed in the dosing suspension. The reviewers concluded that overall, the stability data indicate that the test compound was stable at ambient temperature for up to 8 days.

B. MATERNAL TOXICITY

1. Mortality - One female from 700 mg/kg/day group was found dead on GD 10. Treatment-related death also occurred at 800 mg/kg/day in a preliminary five-day study. Since spontaneous deaths are uncommon in this strain of rat the death of high-dose female in this study was considered to be compound-related.

2. Clinical observations - Compound-related increased incidences of dark feces (daily) and dried brown staining primarily around mouth (within 1-hour

post-dosing) were noted in both treatment groups (11/12 and 1/12 females at 100 mg/kg/day; 12/12 and 11/12 females at 700 mg/kg/day, respectively; data not shown). These findings occurred in a non-dose related fashion and were considered to be test article-related but not necessarily as signs of toxicity. Other clinical signs including hair loss, scabbing, decreased defecation, brown urine and salivation occurred at low frequency and sporadically and therefore, were not considered to be treatment-related.

3. Body weight - Body weights were comparable among all groups. The body weight gain data are summarized in Table 2. Compound-related decreases in body weight gain was noted at 700 mg/kg/day. At this dose level, a transient decrease in body weight gain was observed for GD 6-9 (17%); this decrease was caused by lower body weight gains on GD 6-7 (1 g vs 3 g in controls) and GD 7-8 (gain of 0 g vs 5 g in controls). These differences from the control group were not statistically significant. During GD 9-12, 12-16 and 16-20, mean maternal body weight gains in these groups were comparable to the control values. The mean uterine weights were comparable with controls. However, mean corrected body weight gain was lower (11%) than the control group. This decrease was attributed to increased post-implantation loss. No treatment-related effects on mean body weight gains were noted in the 100 mg/kg/day dose groups.

4. Food consumption - Food consumption data are summarized in Table 3. A compound-related significant decrease in food consumption was observed at 700 mg/kg/day only during GD 6-9 (17%; $p<0.05$). This decrease was caused by significant decrease in food consumption during GD 6-7 (25%; $p<0.01$) and GD 7-8 (21%; $p<0.05$). For the remainder of the treatment period (GD 9-12 and 12-16) and during GD 6-16 and 16-20, the food consumption in this dose group was comparable to the control group. No compound-related effects were noted at 100 mg/kg/day.

5. Gross Pathology - One dam that died on GD 10 had distended urinary bladder and dilated renal pelvis. No compound-related gross pathology findings were noted in the surviving animals at scheduled necropsy. Incidental observations included one control dam with a nodule in the mesentery attached to the urogenital muscle wall

TABLE 2. Mean Body Weight Gain (g ± S.D.)[a]

Study Period in Days	Dose Groups (mg/kg/day)		
	0	100	700
Pre-treatment			
0–6	38 ± 10	42 ± 9	35 ± 8
Treatment			
6–7	3 ± 4	1 ± 8	1 ± 5
7–8	5 ± 4	7 ± 3	0 ± 8
6–9	11 ± 5	13 ± 8	8 ± 10 (17%)b
9–12	17 ± 5	18 ± 8	18 ± 15
12–16	36 ± 4	34 ± 8	36 ± 6
6-16	64 ± 6	65 ± 11	62 ± 14
Entire Study Duration			
0-20	171 ± 10	173 ± 19	163 ± 20 (11%)
Corrected Body Weight Change	82 ± 12	87 ± 12	73 ± 11

[a]Data were extracted from Study No. WIL-157014, Table 5 and 6; none significantly different from control
b Percent lower than control

TABLE 3. Mean Food Consumption (g/animal/day ± S.D.)[a]

Study Period in Days	Dose Groups (mg/kg/day)		
	0	100	700
Pre-treatment			
0–6	24 ± 2	26 ± 2	23 ± 2
Treatment			
6–7	24 ± 4	23 ± 6	18 ± 5** (25%)
7–8	24 ± 3	27 ± 3	19 ± 8* (21%)
6–9	24 ± 3	26 ± 3	20 ± 5* (17%)
9–12	26 ± 1	27 ± 3	25 ± 4
12–16	27 ± 2	30 ± 2	27 ± 3
6-16	26 ± 2	28 ± 2	25 ± 2
Entire Study Duration			
0-20	26 ± 2	28 ± 2	25 ± 1

[a]Data were extracted from Study No. WIL-157014, Table 7
[b] Percent lower than controls

*Significantly different from control ($p<0.05$).
**Significantly different from control ($p<0.01$).

6. Cesarean section Data - Data are summarized in Table 4. Compound-related increase in post-implantation loss was noted at 700 mg/kg/day. Mean post-implantation loss increased significantly ($p<0.05$) compared to concurrent as well as historical control values (1.5 losses/dam versus 0.3/dam and 1.4/dam (maximum), respectively). On a percentage basis, the mean percentage of post-implantation loss per litter in the 700 mg/kg/day group (8.5%) also exceeded the concurrent and historical control values (1.5% and 5.6%, respectively). Thus, increased post-implantation loss was considered to be treatment-related. Number of corpora lutea and implantation sites, number of live fetuses/litter or liter size, fetal sex ratio and fetal body weights were unaffected by the treatment. The increase in mean number of resorptions/dam was attributed to early resorptions and therefore, was not treatment-related. No adverse effects were noted at 100 mg/kg/day.

C. DEVELOPMENTAL TOXICITY

There were no fetal external malformations and variations noted in any dose group.

TABLE 4. Cesarean Section Observations[a]

Parameter	Dose Level (mg/kg/day) 0	100	700
No. animals assigned	12	12	12
No. animals mated	12	12	12
No. animals pregnant	12	11	11
Pregnancy rate (%)	100	92	92
Maternal wastage			
No. died/nonpregnant	0	0	0
No. died/pregnant	0	0	1
No. nonpregnant	0	1	1
No. aborted	0	0	0
Total corpora lutea	203 (12)b	204 (11)	179 (10)
Corpora lutea/dam	16.9 ± 1.7c	18.5 ± 2.7	17.9 ± 2.3
Total implantations	189	179	169
Implantations/dam	15.8 ± 1.5	16.3 ± 3.3	16.9 ± 2.8
Total live fetuses	186	168	154
Live fetuses/dam	15.5 ± 1.4	15.3 ± 2.9	15.4 ± 2.7
Total resorptions	3	11	15
Early	3	11	14
Late	0	0	1
Resorptions/dam[d]	0.12 ± 0.4	0.5 ± 0.9	0.8 ± 1.4
Total dead fetuses	0	0	0
Dead fetuses/dam	0	0	0
Fetal weight/litter (g)	3.7 ± 0.2	3.7 ± 0.2	3.8 ± 0.2
Mean Preimplantation loss (%)	14.0 (6.7)	25.0 (12)	10.0 (5.8)
Mean Postimplantation loss (%)	3.0 (1.5)	11.0 (5.7)	15.0*(8.5)
Sex ratio (% male)	46	47	54

aData were extracted from Study No. WIL-157014, Tables 1 , 9 and 10, and pp. 34 and 47.
bNumber of pregnant dams in each group
cMean ± S.D.
dCalculated by the Reviewers

*$p < 0.05$

TABLE 5. Fetal External and Visceral Examination[a]

Findings[b]	Dose Level (mg/kg/day) 0	100	500
External Anomalies			
No. fetuses (litters) examined	186 (12)	168 (11)	154 (10)
Total No. fetuses (litters) with external malformations	0	0	0
Total No. fetuses (litters) with external variations	0	0	0

[a]Data were extracted from Study No. WIL-157014, Tables 10, 12, 13 and 14; pp. 51-54

III. DISCUSSION:

A. MATERNAL TOXICITY: Compound-related maternal toxicity was observed at 700 mg/kg/day and was manifested as death of one dam on GD 10. Additionally, decreased body weight gain and food consumption occurred in dams at 700 mg/kg/day during GD 6-9 of the dosing period.

Based on these results, the maternal LOEL was 700 mg/kg/day; the NOEL was 100 mg/kg/day.

B. DEVELOPMENTAL TOXICITY:

1. Deaths/Resorptions: At 700 mg/kg/day, treatment-related increase in postimplantation loss was observed. The mean percentage of post-implantation losses exceeded the concurrent and the historical values and, therefore, this finding was considered to be indicative of a developmental effect.

2. Altered Growth: No effects on the fetal body weights were seen.

3. Developmental Anomalies: No fetal external malformation and variations were seen.

Based on the finding of increased post-implantation loss at 700 mg/kg/day, the developmental LOEL and NOEL were 700 and 100 mg/kg/day, respectively.

C. STUDY DEFICIENCIES:

- The preliminary five-day toxicity study was conducted on 2 nonpregnant animals/group. Due to insufficient number of animals used it is difficult to assess compound-related effects. Although the result of preliminary study revealed no treatment-related effects at doses lower than 800 mg/kg/day, it was not intended to assess developmental toxicity. In the main study, only two dose levels were used. Use of three dose levels including an intermediate dose and sufficient number of pregnant animals would have allowed assessment of dose-response for maternal and developmental toxicity. No attempts were made to examine the treatment-related

visceral and skeletal effects in fetuses. The study should be repeated using at least 12 pregnant rats per dose with an intemediate dose level and should include a detailed examination of fetuses.

D. CORE CLASSIFICATION: Acceptable (non-guideline)

012283

KOMEEN Developmental Toxicity

Primary Review by: Sanjivani B. Diwan, Ph.D. Sanji Diwan Date 7/16/97
Review Section I, Toxicology Branch II/HED (7509C)
Secondary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson Date 7/16/97
Review Section I, Toxicology Branch II/HED (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Teratology - Developmental Toxicity/Rat

DP BARCODE: D230571; D230574

P. C. CODE: 024407

MRID No: 44127506

SUBMISSION No.: S512801; S512802

TEST MATERIAL: Komeen

CAS No.: 13426-91-0

SYNONYMS: Copper ethylenediamine complex; Bisethylediamine copper chelate

STUDY REPORT NUMBER: WIL-157014

SPONSOR: Griffin Corporation, Valdosta, GA

TESTING FACILITY: WIL Research Laboratories, Inc., Ashland, OH

TITLE OF REPORT: A Developmental Toxicity Screening Study of K-Tea and Komeen in Rats

AUTHOR: D. G. Stump

REPORT ISSUED: August 9, 1996

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID# 44127507) Komeen (8.0% Copper as a.i.) was administered in water (5 mL/kg) to two groups of twelve female Sprague-Dawley rats by gavage at dose levels of 0; 100, or 500 mg/kg/day from gestational days 6-15, inclusive.

Maternal toxicity was observed at 500 mg/kg/day, was manifested as transient reduction in body weight gain accompanied by decrease in food consumption during gestation (day 6-7). However, no frank maternal toxicity was observed. **The maternal NOEL/LOEL is 500 mg/kg/day, based on transient decrease in body weight gain and food consumption. The maternal NOEL is 100 mg/kg/day.**

No developmental toxicity was observed. **The NOEL for developmental toxicity is 3500 mg/kg/day. The developmental LOEL was not established.**

This study is classified as **Unacceptable** (nonguideline) because the highest dose failed to produce frank maternal toxicity. The lack of developmental toxicity at 500 mg/kg/day (based on limited observations) does not guarantee safe exposure at >500 mg/kg/day. The study should be repeated at two higher dose levels including limit dose (1,000 mg/kg/day) to assess dose-response. Additionally, a detailed fetal examination should be conducted to fully investigate the developmental potential of the test compound.

COMPLIANCE: The signed statements of No Data Confidentiality Claim, Compliance with EPA, OECD and Japanese MAFF GLPs as well as Quality Assurance statements were provided. No Flagging Criteria statement was provided.

I. MATERIALS AND METHODS

A. MATERIALS:

Test Material: Komeen
Synonym: Copper ethylenediamine complex
Purity: 8.0% copper (doses not adjusted for % a.i.)
Lot Number: 1150007149
Description: Dark blue liquid
Storage Conditions: Under ambient conditions

B. Vehicle: Deionized water

D. Test Animals: Rat
Species: Sprague-Dawley Crl:CD® BR
Source: Charles River Breeding Laboratories, Inc., Portage, MI.
Age: Females - approximately 12.5 weeks at initiation of dosing
Weight: Females - 226-276 g at initiation of dosing
Housing: Individually in wire-mesh cages
Environmental Conditions:
Temperature: target of 72±4°F
Relative humidity: target of 30-70%
Photoperiod: 12 hours light/dark
Air changes: Not reported
Food and Water: Purina Certified Rodent Chow #5002 and water *ad libitum*
Acclimation Period: 13 days
Males used: Untreated, sexually mature males from the same source and strain

B. PROCEDURES AND STUDY DESIGN:

This study was designed to assess developmental toxicity of Komeen administered daily by gavage to rats on gestation days (GDs) 6 through 15, inclusive.

1. Mating: Following acclimation period, females were mated with males of the same strain and source. Females were checked daily for the presence of vaginal sperm or a copulatory plug. The day on which mating was confirmed was designated day 0 of gestation.

2. Animal Assignment and dose selection is presented in table 1. Animals were consecutively assigned to three groups containing twelve rats each by the randomization procedure as shown below.

TABLE 1.. Animal Assignment

Test Group	Dose Level (mg/kg/day)	Number Assigned per Group
Control	0	12
Low dose	100	12
High dose	500	12

3. Dose Selection Rationale: The dose levels were selected based upon the results of preliminary five-day toxicity study by the sponsor. In this study Komeen was administered to groups of two female Sprague-Dawley Crl:CD BR rats at oral doses of 25, 50, 100, 200 and 400 mg/kg/day for five consecutive days. No mortalities were observed. Clinical signs consisting of decreased defecation and soft stools were sporadically seen in dosed animals. Mean body weights and body weight gains were comparable to control group values.

4. Dosing: All doses were in a volume of 5 ml/kg of body weight/day prepared twice during the study. The appropriate amounts of the test material were weighed into precalibrated containers and a sufficient amount of deionized water, was added to each container. The preparations were stirred on a magnetic stir plate throughout the sampling and dosing procedures. The formulations were divided into aliquots for daily dose administration and stored at room temperature. The individual dosages were based on the most recently recorded body weights to provide the correct mg/kg dose. One set of each sample was analyzed to determine the homogeneity. The stability of the second set of samples was determined over 8 day period.

C. OBSERVATIONS:

1. Maternal Observation and Evaluations - The animals were checked for signs of toxicity approximately one hour following dosing. Individual clinical observations

were recorded daily prior to dosing and during the dosing period (GD 0-20). Body weights were recorded on gestation day (GD) 0, 6 to 16 (daily) and 20; group mean body weight changes were calculated for each corresponding intervals and also for intervals 6-9, 9-12, 12-16, 6-16 and 0-20. In addition, corrected body weight gains were determined at necropsy. Food consumption was recorded for the following periods: GD 0, 6-16 (daily) and 20. Examinations at sacrifice consisted of:

- Gross pathology observations for evidence abnormalities
- Individual uterine weights
- Number of corpora lutea
- Number of implantation sites; the uteri from apparently nonpregnant animals were placed in 10% ammonium sulfide solution using the Salewski (1964) technique to detect early implantation loss
- Numbers of resorptions (early and late) and live and dead fetuses
- Number and distribution of fetuses in each uterine horn

2. Fetal Evaluations - The fetuses were examined in the following manner:

- Individual fetal weight and sex
- External malformation and variations
- Crown-rump measurements for late resorptions

3. Historical control data were provided to allow comparison with concurrent controls.

D. STATISTICAL ANALYSIS: The following methods were used.

- Maternal and fetal body weight and body weight change, uterine and net body weight changes, food consumption, number of corpora lutea, total number of implantations and viable fetuses--One-way ANOVA with Dunnett's test

- Early and late resorptions, dead fetuses, postimplantation losses--Mann-Whitney U-test

- Fetal sex ratios--Chi-square test with Yates' correction factor

- Malformations and variations-- Fischer's Exact test

II. RESULTS

A. TEST MATERIAL ANALYSES

The concentration analysis for the first set of samples for the two dose groups indicated values within ±5% of the nominal concentration (105% and 100% at 100 and 500 mg/kg/day, respectively). Stability analyses conducted on the second set of test samples revealed values within ±2% of target, thus showing no degradation. The test compound was homogeneously distributed in the dosing suspension. The reviewers concluded that overall, the stability data indicated that the test compound was stable at ambient temperature for up to 8 days.

B. MATERNAL TOXICITY

1. Mortality - No mortality was observed.

2. Clinical observations - Compound-related increased incidences of dark feces (observed daily) and dried brown staining primarily around mouth (noted within 1-hour post-dosing) in both the treatment groups (6/11 and 11/12 females at 100 mg/kg/day; 11/11 and 9/12 females at 500 mg/kg/day, respectively). Because of lack of effect on the general health of the animals, these findings although test article-related, were not considered to be toxic effects.

3. Body weight - Body weights were comparable among treated and control groups. Body weight gain data are summarized in Table 2. Compound-related decreases in body weight gain were observed at 500 mg/kg/day. At this dose level, significant decreases in body weight gain were observed for GD 6-9 (36%; nonsignificant). This was due to weight loss on GD 6-7 (-2 g versus +3 g in controls) During remainder of the treatment period (GD 9-12, 12-16 and 16-20), post-treatment period (GD 16-20) and for the entire treatment period (GD 6-16), mean maternal body weight gains and corrected body

weight changes in treated groups were comparable to the control values. No treatment-related effects on mean body weight gains were noted in the 100 mg/kg/day dose groups.

TABLE 2. Mean Body Weight Gain (g ± S.D.)[a]

Study Period in Days	Dose Groups (mg/kg/day)		
	0	100	500
Pre-treatment			
0-6	38 ± 10	35 ± 10	42 ± 7
Treatment			
6-7	3 ± 4	5 ± 3	-2 ± 6
7-8	5 ± 4	4 ± 5	6 ± 5
6-9	11 ± 5	15 ± 4	7 ± 6 (36%)[b]
9-12	17 ± 5	18 ± 4	15 ± 9
12-16	36 ± 4	38 ± 8	36 ± 6
6-16	64 ± 6	71 ± 10	57 ± 8
Entire Study Duration			
0-20	171 ± 10	179 ± 9	173 ± 15
Corrected Body Weight Change	82 ± 12	82 ± 11	81 ± 11

aData were extracted from Study No. WIL-157014, Table 5 and 6; none significantly different from control
b Percent lower than control

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4. Food consumption - Food consumption data are summarized in Table 3. A compound-related decrease) in food consumption was observed at 500 mg/kg/day during GD 6-9 (13%; nonsignificant). This was due to significant reduction in food consumption during GD 6-7 (37%; $p<0.01$). For the remainder of the treatment period (GD 9-12 and 12-16) and during GD 6-16 and 16-20, the food consumption in this dose group were comparable to the control group. No compound-related effects were noted at 100 mg/kg/day.

5. Gross Pathology - No compound-related gross pathology findings were noted in animals at scheduled necropsy.

7. Cesarean section Data - Data are summarized in Table 4. There were no compound-related effects on the number of corpora lutea and implantation sites, mean post-implantation loss, number of live fetuses/litter or liter size, fetal sex ratio and fetal body weights observed in any dose group. Increase in the number of corpora lutea at 100 mg/kg/day was considered to be incidental.

C. DEVELOPMENTAL TOXICITY

There were no fetal malformations and variations were noted in any dose group.

TABLE 3. Mean Food Consumption (g/animal/day ± S.D.)[a]

Study Period in Days	Dose Groups (mg/kg/day)		
	0	100	500
Pre-treatment			
0-6	24 ± 2	24 ± 2	25 ± 3
Treatment			
6-7	24 ± 4	24 ± 1	15 ± 3**b (37%)
7-8	24 ± 3	25 ± 2	22 ± 4 (8%)
6-9	24 ± 3	25 ± 1	21 ± 3b (13%)
9-12	26 ± 1	27 ± 2	25 ± 2
12-16	27 ± 2	29 ± 3	27 ± 2
Entire Study Duration			
6-16	26 ± 2	27 ± 2	24 ± 2
0-20	26 ± 2	27 ± 1	26 ± 2

[a]Data were extracted from Study No. WIL-157014, Table 7
[b]Body weight gain during GD 6-7 and GD 6-9 was 37% and 13% lower than the control group, respectively.
**Significantly different from control ($p<0.01$).

TABLE 4. Cesarean Section Observations[a]

Parameter	Dose Level (mg/kg/day) 0	100	500
No. animals assigned	12	12	12
No. animals mated	12	12	12
No. animals pregnant	12	11	12
Pregnancy rate (%)	100	92	100
Maternal wastage			
No. died/nonpregnant	0	0	0
No. died/pregnant	0	0	0
No. nonpregnant	0	0	0
No. aborted	0	0	0
Total corpora lutea	203 (12)b	232 (11)	233 (12)
Corpora lutea/dam	16.9 ± 1.7c	21.1 ± 3.2**	19.4 ± 2.5
Total implantations	189	196	198
Implantations/dam	15.8 ± 1.5	17.8 ± 2.6	16.5 ± 1.0
Total live fetuses	186	192	190
Live fetuses/dam	15.5 ± 1.4	17.5 ± 2.3	15.8 ± 0.8
Total resorptions	3	4	8
Early	3	4	8
Late	0	0	0
Resorptions/dam[c]	0.1 ± 0.4d	0.2 ± 0.5	0.3 ± 0.5
Total dead fetuses	0	0	0
Dead fetuses/dam	0	0	0
Fetal weight/litter (g)	3.7 ± 0.2	3.7 ± 0.2	3.8 ± 0.2
Preimplantation loss (%)	4.0 (6.7)	36.0 (14.6)	35.0 (14.0)
Postimplantation loss (%)	3.0 (1.5)	4.0 (1.8)	8.0 (4.0)e
Sex ratio (% male)	46	54	41

aData were extracted from Study No. WIL-157014, Tables 1, 9 and 10, and pp. 34 and 47.
bNumber of pregnant dams in each group
cMean ± S.D.
dCalculated by the Reviewers
eIncidence in historical control = 6%

**Significantly different from control ($p < 0.01$)

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TABLE 5. Fetal External and Visceral Examination[a]

Findings[b]	Dose Level (mg/kg/day) 0	100	500
External Anomalies			
No. fetuses (litters) examined	186 (12)	192 (11)	190 (12)
Total No. fetuses (litters) with external malformations	0	0	0
Total No. fetuses (litters) with external variations	0	0	0

[a]Data were extracted from Study No. WIL-157014, Tables 10, 12, 13 and 14; pp. 51-54

TABLE 5. Fetal External and Visceral Examination[a]

Findings[b]	Dose Level (mg/kg/day) 0	100	500
External Anomalies			
No. fetuses (litters) examined	186 (12)	192 (11)	190 (12)
Total No. fetuses (litters) with external malformations	0	0	0
Total No. fetuses (litters) with external variations	0	0	0

[a]Data were extracted from Study No. WIL-157014, Tables 10, 12, 13 and 14; pp. 51-54